Analysis of cellular fatty acids for identification of microorganisms

CCUG CFA-FAME

Introduction

Since the early 1970s, analysis of cellular fatty acids between 9 and 20 carbons in length have been used as an aid in rapid identification and classification of microorganisms. Gas chromatography of fatty acid methyl esters (FAME) has been the analytical method of choice. Advancements in GC technique and computerized analysis of analytical data have enabled development of routine methods for microbial fatty acid analysis. There is also one commercial system available, the MIDI Sherlock MIS (MIDI Inc, 125 Sandy Drive, Newark De 19713, USA, www.midi-inc.com), which was originally introduced by the Hewlett-Packard Company in 1985. The system used by CCUG, introduced in 1988, was developed locally using the data handling capabilities of the HP 3366A integrator and computer software for database management.

Strains are cultivated under optimal growth conditions:

- Nonfastidious organisms are cultivated on our standard medium: Columbia II agar base (BBL 4397596) with 5% horse blood, aerobically for 16-48h. at 37°C
- Fastidious organisms are cultivated on Chocolate agar (complex formulation), either in CO_2, microaerophilic or anaerobic for 16h to 72h at the optimum temperature

Gas Chromatographic analysis of the samples:

Sample processing and GC setup as described in MIDI Technical Note #101:

- Harvesting, saponification, methylation, extraction, wash.
- Analysis on a Hewlett Packard gas chromatograph (HP 5890A), autosampler (HP 7673), integrator HP 3396A. Injection on a column (25m x 0.2mm x 0.33 um HP-5 crosslinked 5% Ph Me silicone [= 5% diphenyl- and 95% dimethyl-polysiloxane]).
- After integration, peak data are handled by software in the integrator (Klas Lindgren, personal communication). After correction of the area% with Agilent/MIS FAME standard as reference, retention time data are converted to Equivalent Chain Length (ECL) values. Correlating calculated ECL's with known ECL values in the Peak Naming Table identifies peaks. A report is printed on the integrator, and the result is transferred to the computer for further processing.

Database and numerical analysis:

Information is handled by a database management software (Knowledgeman/Guru Expert Systems Management, MDBS, Inc. P.O. Box 2438, Lafayette, Indiana 47996-2438, USA), containing a library of data from known or reference strains. Comparison with the library is performed by a software package (CCUG - J.Drews and F.Acosta) using the Eerola-Lehtonen-formula (Ref. 262) for probabilistic calculations and drawing of dendrograms from cluster analysis.

CCUG system vs MIDI system

While the CCUG system uses one single library for fatty acids and strains, the MIDI software is divided into several libraries, depending on organism (aerobe, anaerobe or yeast) and culturing conditions, which in some cases will enable improved identification of the fatty acids, since some overlapping occurs. Also, the strain libraries are individual for each system.

Reference 262 for strain matching and cluster analysis:

E. Eerola and O. Lehtonen. Optimal data processing procedure for automatic bacterial identification by gas-liquid chromatography of cellular fatty acids. J. Clin. Microbiol. 26: 1745-1753, 1988